APPENDIX A

Proposed Count

A composition comprising at least two probes, each labeled with a distinguishable label, for detecting a chromosomal aberration involving the BCR and ABL genes, said chromosomal aberration having an ABL gene side and a BCR gene side, wherein one of said probes hybridizes to the ABL gene side of said chromosomal aberration and the other of said probes hybridizes to the BCR gene side of said chromosomal aberration, wherein said probes hybridize to an aberrant chromosome wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis.

APPENDIX B

Exemplary support for new claims in 07/537,305, filed June 12, 1990.[1]

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
1. A composition comprising at least two probes, each labeled with a distinguishable label,	127. A composition comprising at least two probes, each labeled with a distinguishable label,	"In particular, chromosome specific staining reagents are provided which comprise heterogeneous mixtures of nucleic acid fragments, each fragment having a substantial fraction of its sequences substantially complementary to a portion of the nucleic acid for which specific staining is desired - the target nucleic acid, preferably the target chromosomal material. In general, the nucleic acid fragments are labeled by means as exemplified herein and indicated infra." *p. 18, lines 14-20; ¶ 0071.* "Section III *infra* describes methods of rendering the probe visible. Since multiple compatible methods of probe visualization are available, the binding patterns of different components of the probe can be distinguished – for example, by color. Thus, this invention is capable of producing any desired staining pattern on the chromosomes visualized with one or more colors (a multi-color staining pattern) and/or other indicator methods." *p. 36, lines 17-23; ¶ 0137.*	

[1]Applicants reserve the right to supplement this table as necessary or desirable.

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
for detecting a chromosomal aberration involving the BCR and ABL genes, said chromosomal aberration having an ABL gene side and a BCR gene side, wherein one of said probes hybridizes to the ABL gene side of said chromosomal aberration and the other of said probes hybridizes to the BCR gene side of said chromosomal aberration,	for detecting a chromosomal aberration involving the BCR and ABL genes, said chromosomal aberration having an ABL gene side and a BCR gene side, wherein one of said probes hybridizes to the ABL gene side of said chromosomal aberration and the other of said probes hybridizes to the BCR gene side of said chromosomal aberration,	"Specifically herein exemplified are chromosome specific reagents and methods to detect genetic rearrangements . . . that produce the BCR-ABL fusion which is diagnostic for chronic myelogenous leukemia (CML). Such chromosome specific reagents for the diagnosis of CML contain nucleic acid sequences which are substantially homologous to chromosomal sequences in the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 associated with CML. Those reagents produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs. Figure 11 graphically demonstrates a variety of staining patterns which, along with other potential staining patterns, are altered in the presence of a genetic rearrangement, such as, the BCR-ABL fusion." *p 19, line 22 - p 20, line 8; ¶ 0075-6.*	
wherein said probes hybridize to an aberrant chromosome	wherein said probes hybridize to an aberrant chromosome	"Such nucleic acid probes preferably comprise nucleic acid sequences that are substantially homologous to nucleic acid sequences in chromosomal regions that flank . . . breakpoints associated with genetic rearrangements." *p. 19, lines 14-18; ¶ 0073.*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis.	wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis.	"The terms 'staining' or 'painting' are herein defined to mean hybridizing a probe of this invention to a genome or segment thereof, such that the probe reliably binds to the targeted chromosomal material therein and the bound probe is capable of being visualized." *p 36, lines 9-12; ¶ 0137.*	A probe that reliably binds to targeted chromosomal material, and is capable of being visualized, is necessarily "of sufficient length to be specifically detected in cytogenetic analysis."
2. A composition comprising at least two probes for detecting a chromosomal aberration, each probe labeled with a distinguishable label.	128. A composition comprising at least two probes for detecting a chromosomal aberration, each probe labeled with a distinguishable label.	"In particular, chromosome specific staining reagents are provided which comprise heterogeneous mixtures of nucleic acid fragments, each fragment having a substantial fraction of its sequences substantially complementary to a portion of the nucleic acid for which specific staining is desired - the target nucleic acid, preferably the target chromosomal material. In general, the nucleic acid fragments are labeled by means as exemplified herein and indicated infra." *p. 18, lines 14-20; ¶ 0071.* "Section III *infra* describes methods of rendering the probe visible. Since multiple compatible methods of probe visualization are available, the binding patterns of different components of the probe can be distinguished – for example, by color. Thus, this invention is capable of producing any desired staining pattern on the chromosomes visualized with one or more colors (a multi-color staining pattern) and/or other indicator methods." *p. 36, lines 17-23; ¶ 0137.*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
wherein one of said probes hybridizes to a part of the ABL gene on one side of said chromosomal aberration and the other of said probes hybridizes to a part of the BCR gene on the other side of said chromosomal aberration,	wherein one of said probes hybridizes to a part of the ABL gene on one side of said chromosomal aberration and the other of said probes hybridizes to a part of the BCR gene on the other side of said chromosomal aberration,	"Specifically herein exemplified are chromosome specific reagents and methods to detect genetic rearrangements . . . that produce the BCR-ABL fusion which is diagnostic for chronic myelogenous leukemia (CML). Such chromosome specific reagents for the diagnosis of CML contain nucleic acid sequences which are substantially homologous to chromosomal sequences in the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 associated with CML. Those reagents produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs. Figure 11 graphically demonstrates a variety of staining patterns which, along with other potential staining patterns, are altered in the presence of a genetic rearrangement, such as, the BCR-ABL fusion." *p 19, line 22 - p 20, line 8; ¶ 0075-6.*	
wherein said probes hybridize to an aberrant chromosome	wherein said probes hybridize to an aberrant chromosome	"Such nucleic acid probes preferably comprise nucleic acid sequences that are substantially homologous to nucleic acid sequences in chromosomal regions that flank . . . breakpoints associated with genetic rearrangements." *p. 19, lines 14-18; ¶ 0073.*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis	wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis	"The terms 'staining' or 'painting' are herein defined to mean hybridizing a probe of this invention to a genome or segment thereof, such that the probe reliably binds to the targeted chromosomal material therein and the bound probe is capable of being visualized. The terms 'staining' or 'painting' are used interchangeably. The patterns resulting from 'staining' or 'painting' are useful for cytogenetic analysis, more particularly, molecular cytogenetic analysis." *p 36, lines 9-15; ¶. 0137.*	A probe that reliably binds to targeted chromosomal material, and is capable of being visualized, is necessarily "of sufficient length to be specifically detected in cytogenetic analysis."
4. The composition of claim 1 wherein the labels comprise fluorescent labels.	130. The composition of claim 127 wherein the labels comprise fluorescent labels.	"In the examples provided in Section VIII of this application, the probes are labeled such that a dual color fluorescence is produced in the staining pattern of said probes upon in situ hybridization." *p 47, lines 16-19; ¶0167.*	
5. The composition of claim 4 wherein the fluorescent labels are distinguishable under a microscope as different colors.	131. The composition of claim 130 wherein the fluorescent labels are distinguishable under a microscope as different colors.	*See above; also* "Since multiple compatible methods of probe visualization are available, the binding patterns of different components of the probe can be distinguished – for example, by color. Thus, this invention is capable of producing any desired staining pattern on the chromosomes visualized with one or more colors (a multi-color staining pattern) and/or other indicator methods." *p. 36, lines 18-23; ¶ 0137.* "3. Visualization: The slides are mounted fluorescence antifade solution . . . containing 1 mg/ml 4'5-amidino-2-phenylinodle (DAPI) as a counterstain, and examined using a FITC/Texas red double-band pass filter set	FITC and Texas red are art-known fluorescent labels that are distinguishable under a microscope as different colors.

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
		(Omega Optical) on a Zeiss Axioscop." *p. 118, line 24 – p. 119, line 2; ¶ 0349*	
6. The composition of claim 1 wherein the probes hybridize with chromosomal DNA *in situ* in cells.	132. The composition of claim 127 wherein the probes hybridize with chromosomal DNA *in situ* in cells.	"In the examples provided in Section VIII of this application, the probes are labeled such that dual color fluorescence is produced in the staining pattern of said probes upon in situ hybridization (fluorescent in situ hybridization (FISH)." *p. 47, lines 16-19;¶ 0167;* see also *Section IV, p. 74 ("In Situ Hybridization"); ¶ 0247-0264.* "For cells or chromosomes in suspension, a fixation procedure disclosed by Trask, et al. . . . is useful." *p. 76, lines 20-22; ¶ 0254.*	
7. The composition of claim 6 wherein the cells comprise those in interphase of mitotic division.	133. The composition of claim 132 wherein the cells comprise those in interphase of mitotic division.	"Preferably, the staining reagents of the invention are applied to interphase or metaphase chromosomal DNA by in situ hybridization." *p. 23, lines 12-13; ¶ 90.* "The methods and reagents of this invention find a particularly appropriate application in the field of diagnostic cytogenetics, particularly in the field of diagnostic interphase cytogenetics." *p. 46, lines 23-25; ¶ 165.*	It is well known in the art that interphase (and metaphase) are both stages of mitotic cellular division.

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
8. The composition of claim 7 wherein the probes after hybridization are juxtaposed as doublets if a chromosomal aberration is present.	134. The composition of claim 133 wherein the probes after hybridization are juxtaposed as doublets if a chromosomal aberration is present.	Figures 8 and 11c illustrate probes, after hybridization, juxtaposed as doublets when a chromosomal aberration is present. Figure 11 "section c) represents the use of a probe which binds to sequences which come together as a result of the rearrangement and allows for the detection in metaphase and interphase cells. In this case the different sequences are stained with different 'colors.' " *p. 32, lines 11-14; ¶ 0126.*	
10. The composition of claim 8 wherein the chromosomal aberration is further defined as comprising a translocation, said translocation formed by breakpoints which occur on the long arms of chromosomes 9 and 22.	136. The composition of claim 134 wherein the chromosomal aberration is further defined as comprising a translocation, said translocation formed by breakpoints which occur on the long arms of chromosomes 9 and 22.	"Such reagents are exemplary of disease specific, in this case tumor specific, probes which can be labeled, directly and/or indirectly, such that they are visualizable when bound to the targeted chromosomal material, which in the case of CML is the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 known to be associated with CML." *p. 47, lines 12-16; ¶ 0167.*	The designations "9q34" and "22q11" use standard cytogenetic terminology to indicate that the breakpoints in CML occur in the q34 region of the long arm of chromosome 9 and the q11 region of the long arm of chromosome 22. In each case, the letter "q" indicates that the region is part of the long arm of the relevant chromosome.

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
11. The composition of claim 10 wherein the translocation breakpoints are further defined as occurring at the locations designated t(9;22)(q11;q34).	137. The composition of claim 136 wherein the translocation breakpoints are further defined as occurring at the locations designated t(9;22)(q11;q34).	"Such reagents are exemplary of disease specific, in this case tumor specific, probes which can be labeled, directly and/or indirectly, such that they are visualizable when bound to the targeted chromosomal material, which in the case of CML is the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 known to be associated with CML." *p. 47, lines 12-16; ¶ 0167.* "That fusion usually involves a reciprocal translation t(9;22)(q34;q11)." *p. 14, lines 23-24; ¶ 0030.*	
12. The composition of claim 11 wherein the translocation breakpoints are further defined to occur in the BCR and ABL genes respectively, and a fusion gene is formed by the translocation, and said fusion gene comprises portions of the BCR and ABL genes.	138. The composition of claim 137 wherein the translocation breakpoints are further defined to occur in the BCR and ABL genes respectively, and a fusion gene is formed by the translocation, and said fusion gene comprises portions of the BCR and ABL genes.	"The approach in such examples is based on FISH with probes from chromosomes 9 and 22 that flank the fused BCR and ABL sequences in essentially all cases of CML (Figure 8)." *p. 47, line 26 - p. 48, line 2.; ¶ 0168*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
14. The composition of claim 6 wherein the cells comprise a sample of human tissue.	139. The composition of claim 132 wherein the cells comprise a sample of human tissue.	"Sample Preparation: CML-4: Peripheral blood was centrifuged for 5 min. Ten drops of interface was diluted with PBS, spun down, fixed in methanol/acetic acid (3:1), and dropped on slides. CML-2, 3, 7: Five to 10 drops of marrow diluted with PBS to prevent clotting were fixed in methanol/acetic acid and dropped on slides. CML-1,4,5,6: Peripheral blood and/or bone marrow was cultured in RPMI 1640 supplemented with 10% fetal calf serum, an antibiotic mixture . . ., and 1% L-glutamine for 24h. Cultures were synchronized . . . " *p. 116, line 23 - p. 117, line 4; ¶ 0343.*	The passage from Example VIII shown here illustrates the use of the claimed invention on samples of human tissue, *i.e.*, human blood and bone marrow.
15. The composition of claim 14 wherein the human tissue sample comprises peripheral blood.	140. The composition of claim 139 wherein the human tissue sample comprises peripheral blood.	"Sample Preparation: CML-4: Peripheral blood was centrifuged for 5 min. Ten drops of interface was diluted with PBS, spun down, fixed in methanol/acetic acid (3:1), and dropped on slides." *p. 116, lines 23-25; ¶ 0343.*	This passage indicates that the technique is carried out using peripheral blood as the sample.
16. The composition of claim 15 wherein the human tissue sample comprises bone marrow.	141. The composition of claim 139 wherein the human tissue sample comprises bone marrow.	"Sample Preparation: . . . CML-2,3,7: Five to 10 drops of marrow diluted with PBS to prevent clotting were fixed in methanol/ acetic acid (3:1), and dropped on slides." *p. 116, line 23 p. 117, line 1; ¶ 0343.*	This passage indicates that the technique is carried out using bone marrow as the sample.
17. The composition of claim 6 wherein the cells comprise a sample of cultured cells.	142. The composition of claim 131 wherein the cells comprise a sample of cultured cells.	"Sample Preparation: CML-1,4,5,6: Peripheral blood and/or bone marrow was cultured in RPMI 1640 supplemented with 10% fetal calf serum, an antibiotic mixture . . ., and 1% L-glutamine for 24h. Cultures were synchronized . . . " *p. 117, lines 1-4; ¶ 0343.*	This passage indicates that the technique is carried out using cultured human blood and/or bone marrow cells.

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
22. The composition of claim 21 wherein the presence of said fusion gene is diagnostic or prognostic for acute lymphocytic leukemia (ALL).	146. The composition of claim 138 wherein the presence of said fusion gene is diagnostic or prognostic for acute lymphocytic leukemia (ALL).	"the staining patterns produced upon hybridization of nucleic acid probes of this invention to chromosomal material containing a genetic rearrangement associated with ALL is distinctively different from that produced upon hybridization of such probes to chromosomal material containing the BCR-ABL fusion characteristic of CML." *p. 48, lines 11-15; ¶ 0168.* "the diagnosis and study of acute lymphocytic leukemia (ALL) may be accomplished by replacing the BCR probe (PEM12) of section VIII with a probe from the 5' end of the BCR gene. ALL is of particular interest because the Ph^1 chromosome is the most common cytogenetic abnormality in that disease, and the presence of such a chromosome is indicative of a very aggressive neoplasm." *p. 49, lines 7-13; ¶ 0171.*	As can be seen from these passages, it was known in the art at the time the present invention was made that ALL was characterized by a different BCR/ABL translocation than that found in CML. The presently claimed invention provides a method for distinguishing the Ph' fusion gene in CML from that produced in ALL.
23. The composition of claim 21 wherein the presence of said fusion gene is diagnostic or prognostic for chronic myelogenous leukemia (CML).	147. The composition of claim 138 wherein the presence of said fusion gene is diagnostic or prognostic for chronic myelogenous leukemia (CML).	"This invention still further provides methods and reagents for producing staining patterns in a patient who is afflicted with a disease associated genetic rearrangement, such as those associated with the BCR-ABL fusion in CML . . . Such staining patterns can be useful in monitoring the status of such a patient . . . and can be predictive of a disease recurrence for a patient that is in remission." *p. 20, line 25 - p. 21, line 7; ¶ 0080.*	This passage indicates that detecting the presence of the fusion gene in a patient for the first time is diagnostic for CML. Detecting the presence of the fusion gene in a patient known to have CML, during the course of treatment, is prognostic in that it can indicate a possible recurrence, or serve as a measure of the success of ongoing treatment.

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
24. A kit for the detection of chromosomal aberrations,	148. A kit for the detection of chromosomal aberrations,	"This invention also provides for test kits comprising high complexity probes for the detection of genetic rearrangements, and specifically for those producing the BCR-ABL fusion characteristic of CML." *p. 25, lines 14-18; ¶ 0096.*	
comprising a first and second nucleic acid probe, each labeled with a distinguishable label,	comprising a first and second nucleic acid probe, each labeled with a distinguishable label,	"In particular, chromosome specific staining reagents are provided which comprise heterogeneous mixtures of nucleic acid fragments, each fragment having a substantial fraction of its sequences substantially complementary to a portion of the nucleic acid for which specific staining is desired - the target nucleic acid, preferably the target chromosomal material. In general, the nucleic acid fragments are labeled by means as exemplified herein and indicated infra." *p. 18, lines 14-20; ¶ 0071.* "Section III *infra* describes methods of rendering the probe visible. Since multiple compatible methods of probe visualization are available, the binding patterns of different components of the probe can be distinguished – for example, by color. Thus, this invention is capable of producing any desired staining pattern on the chromosomes visualized with one or more colors (a multi-color staining pattern) and/or other indicator methods." *p. 36, lines 17-23; ¶ 0137.*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
said first probe that specifically hybridizes to a part of the ABL gene on one side of said chromosomal aberration and said second probe that specifically hybridizes to a part of the BCR gene on the other side of said chromosomal aberration,	said first probe that specifically hybridizes to a part of the ABL gene on one side of said chromosomal aberration and said second probe that specifically hybridizes to a part of the BCR gene on the other side of said chromosomal aberration,	"Specifically herein exemplified are chromosome specific reagents and methods to detect genetic rearrangements . . . that produce the BCR-ABL fusion which is diagnostic for chronic myelogenous leukemia (CML). Such chromosome specific reagents for the diagnosis of CML contain nucleic acid sequences which are substantially homologous to chromosomal sequences in the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 associated with CML. Those reagents produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs. Figure 11 graphically demonstrates a variety of staining patterns which, along with other potential staining patterns, are altered in the presence of a genetic rearrangement, such as, the BCR-ABL fusion." *p 19, line 22 - p 20, line 8; ¶ 0075-6.*	
wherein said probes hybridize to an aberrant chromosome	wherein said probes hybridize to an aberrant chromosome	"Such nucleic acid probes preferably comprise nucleic acid sequences that are substantially homologous to nucleic acid sequences in chromosomal regions that flank . . . breakpoints associated with genetic rearrangements." *p. 19, lines 14-18; ¶ 0073.*	

APPENDIX B

CLAIMS – US 6,025,126	PENDING CLAIMS	EXEMPLARY SUPPORT IN SPEC.	COMMENTS
wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis.	wherein said probes are of sufficient length to be specifically detected in cytogenetic analysis.	"The terms 'staining' or 'painting' are herein defined to mean hybridizing a probe of this invention to a genome or segment thereof, such that the probe reliably binds to the targeted chromosomal material therein and the bound probe is capable of being visualized." *p 36, lines 9-12; ¶ 0137.*	A probe that reliably binds to targeted chromosomal material, and is capable of being visualized, is necessarily "of sufficient length to be specifically detected in cytogenetic analysis."
25. The composition of claim 1 wherein the aberrant chromosome is the Philadelphia chromosome.	149. The composition of claim 127 wherein the aberrant chromosome is the Philadelphia chromosome.	"Fusion of the proto-oncogene c-ABL from the long arm of chromosome 9 with the BCR gene of chromosome 22 is a consistent finding in CML. That genetic change leads to formation of a BCR-ABL transcript that is translated to form a 210 kd protein present in virtually all cases of CML. In 90% of the cases, the fusion gene results from a reciprocal translocation involving chromosomes 9 and 22 producing a cytogenetically distinct small acrocentric chromosome called the Philadelphia (Ph^1) chromosome, Fig. 8." *p. 17, lines 1-8; ¶ 0068.* "Particularly described herein is the application of chromosome specific reagents and methods for detecting genetic rearrangements that produce the BCR-ABL fusion associated with CML." *p. 47, lines 9-11; ¶ 0167.*	